

The Whole Body Autoradiographic Studies on the Distribution of Radioisotopes. XXVIII

Distribution of Radioactivity in Mice after Oral Administration of Tritium Labelled O-Dibutyrylated Trimetoquinol (BAQ-509)

Minezo OTSUKA, Mari SAKUMA and Yoshishige SATO

Biological Research Laboratory, Tanabe Seiyaku Co., Ltd.

Kawagishi, Toda-shi, Saitama

Received October 27, 1971

The distribution patterns of tritiated trimetoquinol (AQL-208), its O-dibutyrylated derivative (BAQ-509) and glucuronide of AQL-208 (main metabolite) administered orally were studied in mice by whole body autoradiography.

O-dibutyrylated derivative of trimetoquinol was more quickly absorbed from the gastrointestinal tract than trimetoquinol. But there were not essential differences between the distribution patterns of both compounds. The highest accumulation of radioactivity was recorded in the liver, kidney, lung, gall bladder and urinary bladder. The blood and the connective tissues displayed strong and stable amount of radioactivity. At 24 hr after administration hardly any radioactivity could be observed in the body of the mice. ³H-BAQ-509 hardly passed the placental barrier and the blood-brain barrier.

After oral administration of tritiated glucuronide of AQL-208, it was found that the absorption from the intestine was considerably small. Most part of the absorbed amount was circulated from the liver into intestinal lumen via bile, while minor part of it was excreted via kidney.

Introduction

Trimetoquinol (AQL-208) has been shown to be a potent bronchodilating agent¹⁾⁻⁴⁾. The metabolic fate^{5),6)} and distribution⁶⁾ of tritiated AQL-208 in guinea pigs, mice or rats have been previously reported.

Further pharmacological and pharmacokinetic studies on this drug revealed that its O-dibutyrylated derivative (BAQ-509) has more rapid onset of the bronchodilating action than AQL-208 following oral administration⁷⁾. It seemed, therefore, interesting to compare the distribution of these two compounds in order to see how the differences in chemical structure can be related to that in the distribution pattern and to see how the specific sites of accumulation of the drug can be correlated to the sites of the pharmacological action.

In the present paper, the distribution of

labelled BAQ-509, AQL-208 and its glucuronide which was main metabolite were studied in mice by means of whole body autoradiographic procedure.

Materials and Methods

Labelled compounds

Tritiated trimetoquinol (AQL-208), its O-dibutyrylated derivative (BAQ-509), its glucuronide and ¹²⁵I-human serum albumin were used for the autoradiographic distribution studies.

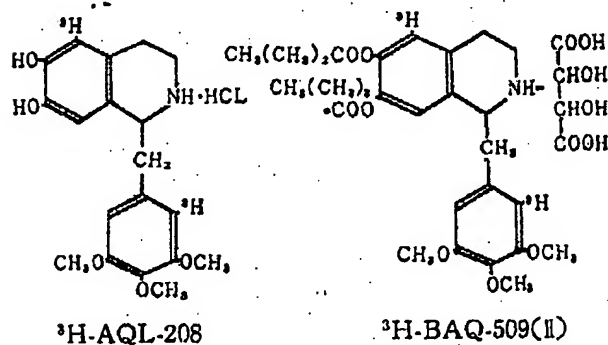
³H-AQL-208 was synthesized by catalytic hydrogenation of dibrominated trimetoquinol (obtained by bromination of trimetoquinol) with 5 Ci of tritium gas as described earlier⁶⁾. ³H-BAQ-509 was synthesized from ³H-AQL-208 by esterification with *n*-butyryl chloride. The compounds indicated over 98 % of chemical and radiochemical purity which was checked by means of thin-layer

BEST AVAILABLE COPY

chromatography. Specific activities obtained were 3.7 mCi/mg for ^3H -AQL-208, and 1.76 mCi/mg for ^3H -BAQ-509, respectively. The glucuronide of ^3H -AQL-208 was isolated from mice urine and purified according to the method given by Satoh, et al.⁹⁾ The total radioactivity of tritiated glucuronide of ^3H -AQL-208 was 296 μCi and its radiochemical purity was higher than 96% as revealed by paper chromatography. ^{125}I -human serum albumin solution was received from the Radiochemical Center, Amersham, England. Specific activity was 1 mCi/ml.

For administration, 1.25 μmole (800 μCi) of the compound, ^3H -AQL-208 or ^3H -BAQ-509, was dissolved in 0.5 ml of physiological saline. The glucuronide of ^3H -AQL-208 was dissolved in 0.5 ml of the saline. The ^{125}I -human serum albumin solution was diluted with saline and the specific activity was 20 $\mu\text{Ci}/\text{ml}$.

The labelled AQL-208 and BAQ-509 are illustrated by the following formulas.



Animal experiments

The ^3H -AQL-208 or ^3H -BAQ-509 was orally given to the mice (800 $\mu\text{Ci}/1.25 \mu\text{mole}/\text{mouse}$, in dose) after fasting for about 16 hours. In the first series of experiments, the labelled compounds were administered orally to the ddY strain male mice weighing about 20 g. These mice were sacrificed at 5, 10, 20 min, 1, 2 and 24 hr. Some mice were killed at 10 and 20 min by bleeding from the femoral artery before freezing. In the second series, the same strain of pregnant mice weighing about 40 g (9~10 days gestation) were frozen 10 and 20 min after administration. In the third series, mice were administered orally with ^3H -BAQ-509 (1.25 μmole , 800 μCi) at

5 min after intravenous injection of non-labelled propranolol hydrochloride (9.65 $\mu\text{mole}/\text{kg}$). These mice were sacrificed with freezing after 10 minutes. In the fourth series, 295 μCi of tritiated glucuronide of AQL-208 was orally given to a mouse, which was sacrificed at 30 min after administration. In the last series, two mice were intravenously injected with 2 μCi of ^{125}I -human serum albumin. These mice were sacrificed the same way 30 sec and 2 min after the injection.

All animals were slightly anesthetized with ether and killed by immersion in acetone kept at -70°C with solid carbon dioxide. The frozen animals were then transferred to a cryostat kept at -15°C .

Autoradiographic procedure

The autoradiographic technique employed was described earlier¹⁰⁾. Sagittal sections, 40 μ thick, of the animals were taken by means of a Leitz 1300 microtome. The sections were dried in a cryostat and brought into contact with Sakura N-type X ray films. After sufficient exposure, the autoradiograms were developed and pictures were made for illustration purposes; thus an increasing degree of whiteness in the illustration corresponds to an increasing concentration of radioactivity.

Results

1. Distribution of ^3H -BAQ-509 and ^3H -AQL-208

The results of the autoradiographic distribution are shown in figures 1~5. The radioactivity in the whole body of mice reached its peak at 5~10 min for BAQ-509 and at 10~20 min for AQL-208 after administration. There are not essential differences between the distribution patterns of both compounds. Accumulation of the absorbed radioactivity was observed in almost all organs and tissues except in the central nervous system. The high levels of radioactivity were found in the blood, lung, liver, kidney and connective tissues. The gall bladder and urinary bladder also showed a

high radioactivity throughout the observation period. At 24 hr after administration the radioactivity could be hardly observed in the body of mice. The detailed distribution in various tissues at different time intervals is given below.

Central nervous system: Accumulation of radioactivity was not observed in the cerebrum, cerebellum and spinal cord throughout the observation period (Fig. 1, 2).

Endocrine glands: The pituitary within 20 minutes after the administration showed concentration which was slightly lower than in the blood. The level of radioactivity in the adrenal was about the same as that in the blood, being higher in medulla than in cortex. The activity in the medulla was never higher than in the blood. The levels in the thymus and thyroid gland were nearly the same as that in the salivary gland. The testis displayed moderately low radioactivity (Fig. 3, 4).

Cardiovascular system: The radioactivity of the blood exceeded that of any other organs except excretory system such as the gall bladder and urinary bladder. The heart muscle had a rather low activity which was retained for two hours after administration (Fig. 1, 2).

Respiratory system: The lung was very highly labelled and the activity remained quite high even 2 hours. The bronchi also retained slightly high activity (Fig. 1, 2, 3). The radioactivity in the trachea was relatively high (Fig. 3B).

Digestive system: The radioactivity in the liver reached its peak at 5~10 min for BAQ-509 and at 10-20 min for AQL-208 and thereafter decreased gradually. In the gall bladder the strong radioactivity was observed throughout the observation period. The salivary glands accumulated a rather low radioactivity (Fig. 1, 2).

Urinary organs: The kidney revealed high concentration during the whole observation periods. The urinary bladder content also showed a very high concentration (Fig. 1, 2).

Muscle and skin: The radioactivity in the muscle was always low, whereas a charac-

teristic high accumulation and retention of radioactivity were observed in the various connective tissue, such as the derma of the skin, costal cartilage, interstitial and subcutaneous connective tissues and so on (Fig. 1, 2).

Placenta and fetus: The concentration of radioactivity was always lower in the foetus than in the placenta. The concentration of radioactivity in the foetus was the same as that in the maternal brain (Fig. 5A, B).

2. Distribution of tritiated glucuronide of AQL-208

As shown in Fig. 6, 30 min after oral administration, the most of the radioactivity was still located in the gastric and intestinal lumen, indicating that the absorption of the compound from the intestine is very limited. An appreciable concentration was shown in the gall bladder and a much lower concentration in the kidney and the urinary bladder. In any other organs or tissues radioactivity was hardly detected (Fig. 6).

3. Distribution of radioactivity in the decapitated mice

The autoradiograms of ^3H -BAQ-509 or ^3H -AQL-208 obtained with mice sacrificed by bleeding were not obviously different from that with non-bled mice (Fig. 7).

4. Distribution of radioactivity in mice pretreated with propranolol

The distribution of ^3H -BAQ-509 did not change by preinjection of propranolol as a β -blocker (Fig. 8).

5. Distribution of ^{125}I -human serum albumin

Two minutes after the intravenous injection almost all the radioactivity appears to be retained in the blood. The level of the radioactivity in the lung was also high. An accumulation may be noticed in the liver, kidney, spleen and adrenal cortex (Fig. 9).

Discussion

From a comparison of the distribution

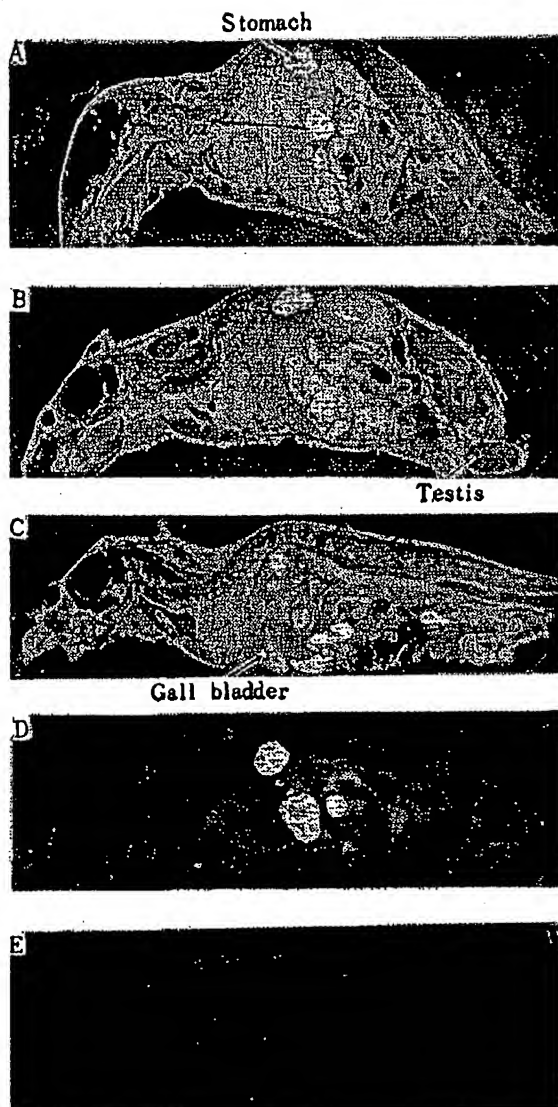


Fig. 1

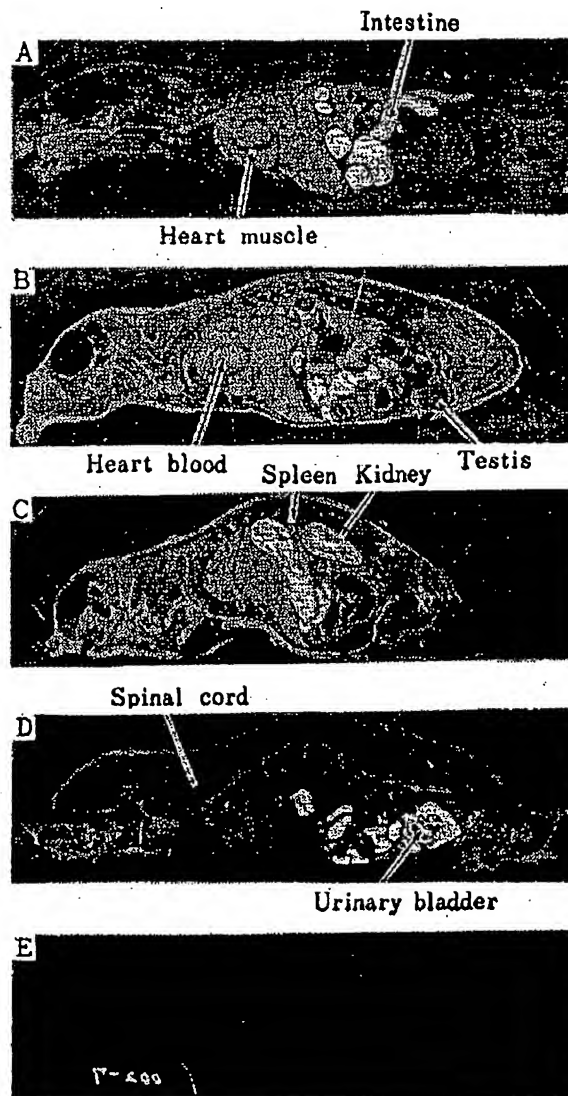


Fig. 2

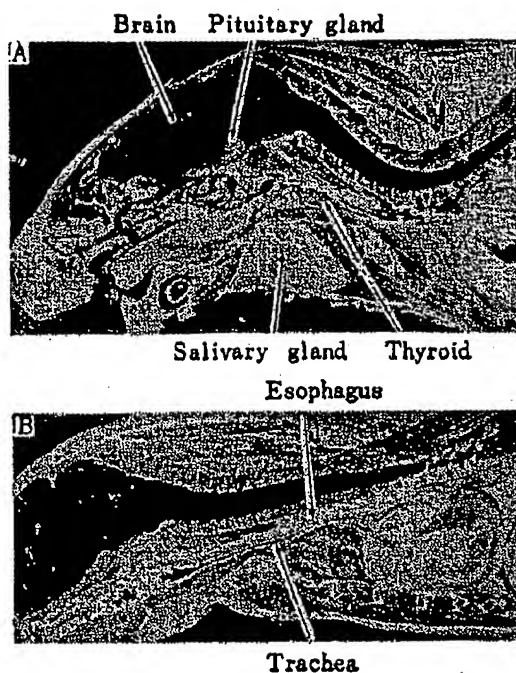
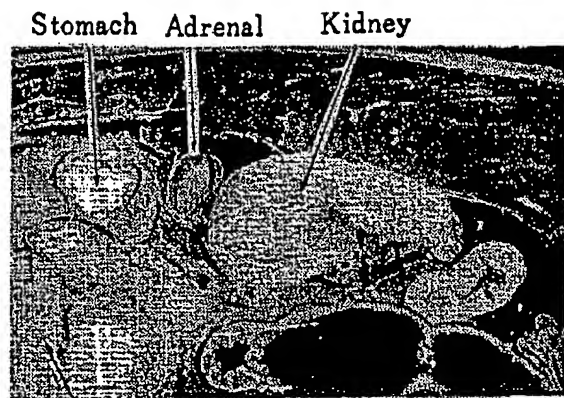


Fig. 3

Fig. 1 Autoradiograms showing the distribution of radioactivity in mice 5 min (A), 10 min (B), 20 min (C), 2 hr (D) and 24 hr (E) after oral administration of ^3H -II-BAQ-509.

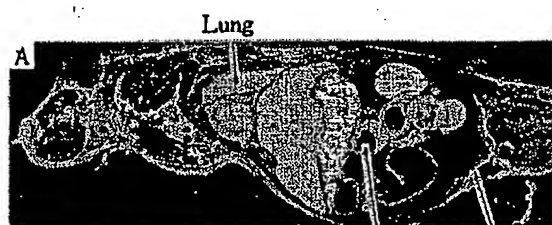
Fig. 2 Autoradiograms showing the distribution of radioactivity in mice 5 min (A), 10 min (B), 20 min (C), 2 hr (D) and 24 hr (E) after oral administration of ^3H -AQL-208.

Fig. 3 Enlargements of autoradiograms showing the distribution in the head, neck and thorax region of mice 10 min (A) and 20 min (B) after oral administration of ^3H -BAQ-509.

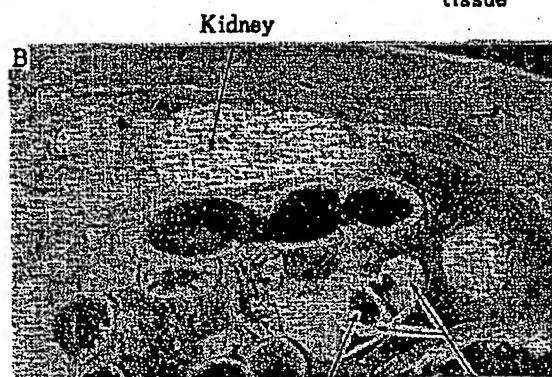


Liver

Fig. 4



A



B

Fig. 5



Gall bladder Urinary bladder

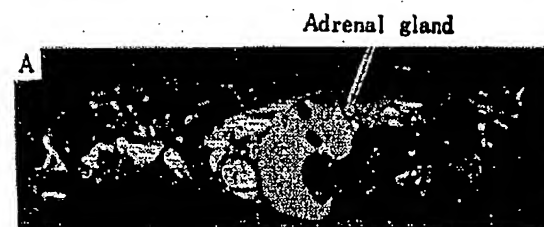
Fig. 6



Fig. 7



Fig. 8



A



B

Liver

Fig. 9

Fig. 4 Enlarged detail of the distribution of radioactivity in a mouse, 20 min after oral administration of ^3H -BAQ-509.

Fig. 5 Autoradiograms showing the distribution of radioactivity in pregnant mice (A and B) 20 min after oral administration of ^3H -BAQ-509.

Fig. 6 An autoradiogram showing the distribution of radioactivity in a mouse 30 min after oral administration of glucuronide of ^3H -AQL-208.

Fig. 7 An autoradiogram showing the distribution of radioactivity at 10 min after oral administration in mice treated with the bleeding from the femoral artery before freezing.

Fig. 8 An autoradiogram showing the distribution of radioactivity at 10 min after oral administration of ^3H -BAQ-509 in a mouse previously treated with propranolol.

Fig. 9 Autoradiograms of mice 30 sec (A) and 2 min (B) after intravenous injection of ^{125}I -human serum albumin.

of the two drugs, O-dibutrylated derivative (BAQ-509) of trimetoquinol, being more lipophilic than trimetoquinol (AQL-208), was more rapidly absorbed from the gastrointestinal tract than AQL-208. Therefore, in the case of ^3H -AQL-208, the peak of radioactivity distributed in the body appeared later and the concentration at the peak point was slightly lower than that of BAQ-509. Throughout observation period, the distribution patterns of both compounds were very similar. This fact assumed that BAQ-509 may be very rapidly hydrolyzed in the liver and changed to activated form (i.e., AQL-208). This assumption was supported from the results obtained biochemically by Sugihara, et al.¹⁹ and pharmacologically by Sato, et al.⁷.

With the exception of the gastrointestinal lumen, the distribution patterns following oral administration of ^3H -AQL-208 and ^3H -BAQ-509 show the same patterns as after intravenous injection¹⁰ of ^3H -AQL-208. The distribution pattern of glucuronide of ^3H -AQL-208, however, differs from that of the former two as shown in Fig. 6. It was found that the absorption from the intestine was considerably small and most part of the absorbed amount was circulated from the liver into the intestinal lumen via bile, minor part of it was excreted via kidney. The radioactivity in the heart blood and lung was little or slight. This suggested that the radioactivity in the blood shown in Fig. 1, 2 may not have any connection with that of the glucuronide, being reabsorbed from intestine.

Since autoradiograms showed that the radioactivity in the blood was high and lasting until 2 hours, the contribution of radioactivity in the blood contained in various tissues should be considered to see the distribution of radioactivity in the tissues. Then, the reflection to the vascularity of the various tissues and the tissue affinity of these compounds were tested by the next two procedures. One is the observation of autoradiograms showing the distribution of ^{125}I -human serum albumin

in animals at early stage after administration. This autoradiograms showed the distribution of blood because serum albumin poorly penetrated into tissues. The other is the observation of the autoradiograms obtained from the decapitated animal. The autoradiograms of ^{125}I -human serum albumin showed high concentration in such tissues as the lung, liver, kidney and spleen (Fig. 9). From the autoradiogram of Fig. 9, the concentration of radioactivity in these tissues shown in Fig. 1 and 2 must be considered the reflection of that in blood. It was also expected that the autoradiographic pictures shown in Fig. 1 and 2 would be more or less different from that in Fig. 7, but the autoradiograms obtained from the decapitated animal were similar to that shown in Fig. 1 and 2. It was due to the fact that the blood still retained in the blood vessel and tissues in the decapitated animal. From these observations, these drugs are high in blood and highly taken up and bounded by some tissues.

In the previous paper¹⁰ on the distribution of the radioactivity in the pregnant mouse following intravenous injection of ^3H -AQL-208, it was reported that the concentration of radioactivity in the placenta was almost the same as in maternal heart blood and the penetration into the foetus was hardly found. In the case of ^3H -BAQ-509, it was examined at the middle stage of gestation of mouse following oral administration but these distributions in the placenta and foetus were very similar to those of ^3H -AQL-208 following intravenous injection even at the earlier observation times. Mayer, et al.¹⁶ reported that the passage through the blood-brain barrier and placental barrier of the basic drugs may be due to its high lipid solubility. Since BAQ-509 was quickly metabolized into AQL-208 in the body, the passage through these barrier became very low. In general, the character of the blood-brain barrier is similar to that of placental barrier¹⁷. As a rare example, Sjöstrand and Schmitterlöw¹⁸ have shown that SK-7 passes the blood-brain barrier rather easily but penetrates the

placental barrier rather poorly. On the contrary, the next two compounds, ^{35}S -glutathion⁽¹¹⁾ and ^3H -SDDS⁽¹²⁾, hardly penetrated into the brain through the blood-brain barrier but passed the placental barrier easily.

When interpreting the autoradiograms, it is important to discuss the distribution of labelled compound in relation to known pharmacological effects. Although the accumulation of radioactivity in an organ may not always indicate the site of pharmacological action, it is of interest that these compounds show a relatively high uptake in the trachea and lung. BAQ-509, AQL-208 and its metabolites did not seem to pass the blood-brain barrier. The pharmacological results⁽¹³⁾ have shown that AQL-208 has no effects on the central nervous system even in high doses. Masuoka, et al.⁽⁹⁾ have reported that the three β -receptor blockers, DCI, Kō 592 and propranolol, show a similar distribution picture. However, the preinjection of propranolol into a mouse 5 min earlier did not change, though some differences did expected, the distribution pattern of ^3H -BAQ-509. For this finding and its interpretation, more detailed macro- and micro-autoradiographic studies are in progress.

Acknowledgements

The authors wish to express their deep gratitude to Dr. K. Abe, Director of this research laboratory, for his interest and encouragement throughout this work. The technical assistance of Miss M. Yaitabashi is gratefully recognized.

References

- 1) Y. Iwasawa and A. Kiyomoto: *Jap. J. Pharmacol.*, **17**, 143 (1967)
- 2) M. Sato, I. Yamaguchi and A. Kiyomoto: *ibid.*, **17**, 153 (1967)
- 3) M. Sato, Y. Iwasawa and A. Kiyomoto: *Folia Pharmacol. Japon.*, **64**, 268 (1968)
- 4) A. Kiyomoto, Y. Iwasawa and S. Horigaya: *Arzneim-Forsch.*, **20**, 46 (1970)
- 5) T. Meshi, M. Otsuka and Y. Sato: *Biochem. Pharmacol.*, **19**, 2937 (1970)
- 6) T. Takahashi and Y. Sato: *Radioisotopes*, **17**, 374 (1968)
- 7) M. Sato and A. Kiyomoto: in preparation (1971)
- 8) C. Satoh, T. Nagao, T. Kono and A. Kiyomoto: *Chem. Pharm. Bull.*, **19**, (4) 667 (1971)
- 9) D. Masuoka and E. Hansson: *Acta Pharmacol. et toxicol.*, **25**, 447 (1967)
- 10) S.E. Sjöstrand and C.G. Schmiterlow: *Arzneim-Forsch.*, **18**, 62 (1968)
- 11) T. Takahashi and Y. Sato: *Radioisotopes*, **17**, 1 (1968)
- 12) M. Sakuma and Y. Sato: *ibid.*, **18**, 143 (1969)
- 13) J. Sugihara and Y. Sato: *Radioisotopes*, **20**, 525 (1971)
- 14) Y. Sato and K. Abe: *Pharmacometrics*, **3**, (1) 1 (1969); **3**, (2) 77 (1969)
- 15) A. Kiyomoto, M. Sato, T. Nagao and H. Nakajima: *Europ. J. Pharmacol.*, **5**, 303 (1969)
- 16) S. Mayer, R. P. Maickel and B. B. Brodie: *J. Pharmacol. exp. Therap.*, **127**, 205 (1959)
- 17) B. B. Brodie, H. Kurz and L. S. Schanker: *J. Pharmacol. exp. Therap.*, **130**, 20 (1960)

要 旨

全身オートラジオグラフィによる放射性化合物の
生体内分布に関する研究 (28報)トリチウム標識 *o*-dibutyl trimetoquinol (BAQ-509) の
経口投与によるマウス生体内分布

大塚 峯三, 佐久間 真理, 佐藤 善重

田辺製薬(株)生物研究所

埼玉県戸田市川岸2-2-50

BAQ-509, AQL-208 およびそのグルクロン酸抱合体の経口投与による生体内分布について全身オートラジオグラフィで検討した。

AQL-208 の *O*-ジブチリル誘導体である BAQ-509 は AQL-208 よりも速やかに消化管から吸収された。しかし両化合物の分布様式には本質的な違いはみられなかった。放射能の高い取込みが見られたのは、肝、腎、胆のうおよび膀胱などであった。また、血液や結合組織にも高く、かつ持続した放射能が見られた。しかし投与後24時間では、放射能はほとんど見いだせなかった。BAQ-509 の胎盤-胎子および血液-脳関門の通過はきわめて少なかった。

両化合物のおもなる代謝物である AQL-208 のグルクロナイドを経口投与したとき、消化管からの吸収はかなり少なく、また吸収されたものの大部分は、胆汁を介して肝から腸管内へ排泄され、吸収されたもののうち少量が腎を経て排泄されるのを認めた。